

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Genichiro SOMA et al
Serial No. : 09/700,713
Filed : November 27, 2000
For : ADDITIVES FOR CRUSTACEAN OR FISH FEEDS AND FEEDS
Art Unit : 1645
Examiner : Khatol S.Shahnan-Shah
Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION

Sir:

I, Genichiro SOMA, a Japanese national, residing at 10-21, Higashitamagawa 1-chome, Setagaya-ku, Tokyo 158-0084 Japan, declares that:

1. I am a professor in Institute for Health Sciences of Tokushima Bunri University, Department of Immunology and Allergy, Nishihama Bouji, Yamashiro-cho, Tokushima, 770-8514, Japan.

2. I am an inventor of the invention of U.S. Patent Application Serial No.09/700,713 filed November 27, 2000 (hereinafter referred to as "the present invention"). I have studied the official action dated August 25, 2004 issued with respect to the present application. I have also read and studied the references cited in this official action.

4. I have conducted the following experiments to show the superiority of the present invention.

EXPERIMENTS

1. To clarify the ability for production of Tumor Necrosis factor (TNF) by various lipopolysaccharides with different molecular weights, the following lipopolysaccharides were administered intradermally into murine abdomen.

(a) Preparation of solutions of lipopolysaccharides for intradermal administration.

Four Salmonella lipopolysaccharides and one E. coli lipopolysaccharides were used as shown in below.

- (1) S.minnesota Wild type (MW = 14000 ~ 30000)
- (2) R5 (MW = about 3600)
- (3) R7 (MW = about 3400)
- (4) R595 (MW = about 2700)
- (5) lipopolysaccharide of E. coli 0127 : B8 (MW = 10000 ~ 60000)

Each of lipopolysaccharides was dissolved in saline solution under sterile conditions at 2mg/ml concentration. Further, solutions having concentrations of 0.2 mg/ml and 0.02 mg/ml were also prepared by dilution of the 2mg/ml solution with addition of saline solution.

(b) Administration of lipopolysaccharide to mouse and collection of serum

Mouse (two or three mouse per group) was intradermally administered 50 μ l of each of the above lipopolysaccharide solutions into abdomen, and only saline solution (for control). After 90 minutes, blood was collected from the mouse, and blood

serum was separated. TNF activities in serum were measured by L929 cell cytotoxicity assay.

(c) Results of the test

The results were shown in Table 1 and Fig.1.

Lipopolysaccharide R5, R7 and R595 having a low molecular weight less than 4000 demonstrated lower TNF inducing activity than lipopolysaccharide of *E. coli* (MW = 10000 ~ 60000) and *S. minnesota* Wild type (MW = 14000 ~ 30000) having a high molecular weight.

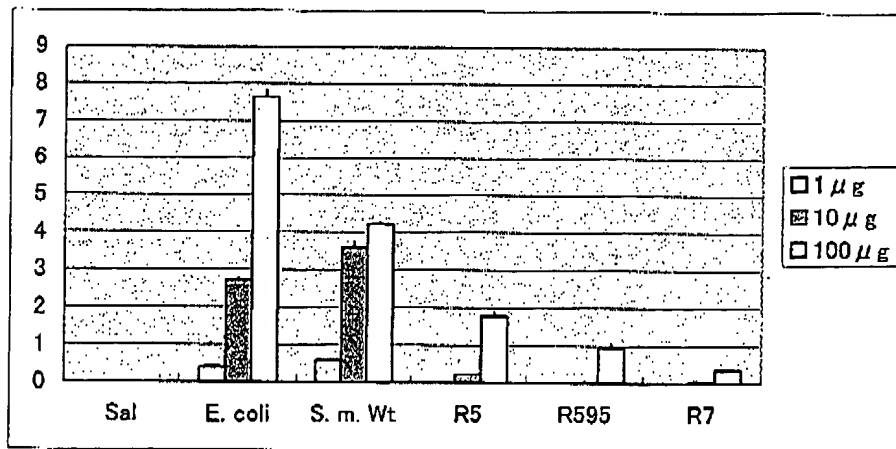
(d) Conclusion

Lower molecular weight of lipopolysaccharides does not correlate to high biological activity of lipopolysaccharide. It depends on the kinds of lipopolysaccharides. In other word, lipopolysaccharide of a low molecular weight does not necessarily have high biological activity depending on the kinds of lipopolysaccharides.

Table 1

sample	dose ($\mu\text{g}/\text{mouse}$)	TNF (u/ml)			average	standard deviation
Saline	0	0	0	0	0	
E.coli	1 μg	0.15	0.45	0.61	0.40	0.13
	10 μg	1.39	4.06		2.73	0.89
	100 μg	3.14	12.15		7.65	3.00
S.minnesota Wild type	1 μg	0.67	0.47	0.64	0.59	0.06
	10 μg	3.51	3.68		3.60	0.06
	100 μg	3.94	4.51		4.23	0.19
R5	1 μg	0	0	0	0	0
	10 μg	0	0.45		0.23	0.15
	100 μg	1.87	1.69		1.78	0.06
R595	1 μg	0	0	0	0	0
	10 μg	0	0		0	0
	100 μg	1.13	0.81		0.97	0.11
R7	1 μg	0	0	0	0	0
	10 μg	0	0.11		0.06	0.04
	100 μg	0.22	0.5		0.36	0.09

Fig. 1



CONSIDERATION OF THE RESULTS

It can be concluded from the above that lipopolysaccharide of a low molecular weight does not correlate to its biological activity.

Namely, lipopolysaccharide of a low molecular weight does not necessarily have high biological activity depending on the kinds of lipopolysaccharides.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed this 17 th day of November, 2004.

A handwritten signature in black ink, appearing to read 'Genichiro SOMA', is written over a horizontal line.

Genichiro SOMA

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.